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Changes in fatty acid composition and conjugated linoleic acid contents of sour dairy products caused by pure cultures

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Abstract. In this research we have investigated the effect of various pure cultures *(Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus lactis subsp. lactis biovar, Lactobacillus diacetilactis, Lactobacillus acidophilus, Bifidobacterium lactis)* on fatty acid composition of soured dairy products (Sana, yoghurt) manufactured using different technologies, with special regard to conjugated linoleic acid (CLA). It was established that the cultures we used and which are also commonly used in the dairy industry, had only a slight effect on fatty acid composition of milk. Although minimal differences were found in case of the individual fatty acids, however, due to the small differences it can be established that the cultures have no influence on nutritional value of milk fat.

Key words and phrases: fatty acid composition, conjugated linoleic acid, dairy products, pure bacteria cultures, different technologies

1 Introduction

Fatty acid composition of milk fat, especially owing to short-chain fatty acids present in relatively big amount, is ideal for the human organism because triacylglycerols containing short-chain fatty acids can be more easily attacked by the digestive enzymes. Milk fat contains relatively small amount of unsaturated fatty acids, despite this it can contain considerable amount of essential fatty acids needed to satisfy the requirements of the human organism and due to its animal origin it contains also the essential arachidonic acid [1]. Milk fat can contain also conjugated linoleic acids (CLA) in considerable quantity, which have according to the latest researches many useful physiological effects. Among others their antioxidant effect, that is they prevent the membranes from the attacks of free-radicals, was proven, consequently they can have significant role in the anti-cancer fight [2, 3].

Composition of dairy products manufactured by adding pure cultures is determined to the greatest extent by the composition of the raw milk, since the cultures produce rather aroma materials and they affect fatty acid composition only to a smaller extent. As regards CLAs some have experienced that as an effect of pure cultures CLA contents of dairy products increased and adding of linoleic acid resulted in a higher CLA contents [4].

It was also established that CLA contents of dairy products manufactured by fermentation could vary, as certain cultures were capable of producing CLA from linoleic acid during the souring [7]. Some reports that CLA contents of cheeses can increase during maturation, others, however, did not establish such relationship [4]. According to most of the authors CLA contents of dairy products depend mainly on CLA contents of the milk used for the production; technological processes can, however, significantly influence CLA contents of the finished product [5, 6]. Some reports that starter cultures can produce CLA in considerable amount, others, however, could not establish such relation. Since until recent times it did not manage to give a definite answer to the question what effect microorganism had on CLA contents of the product, therefore we have decided to examine fatty acid composition and CLA contents of dairy product manufactured from cattle's milk (Sana, yoghurt). By our investigations we would like to draw attention to the outstanding health-protecting effects of soured dairy products.

2 Material and methods

2.1 Used bacteria and the production of soured dairy products

Lactic acid producing *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus diacetilactis* and *Lactobacillus acidophilus* are used for the production of dairy products manufactured by fermentation, while *Lactobacillus lactis subsp. lactis biovar, Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* are used for the production of the very popular yoghurt. Due to proteolitic enzymes of *Lactobacillus delbrueckii subsp. bulgaricus* increase the free amino acid contents, especially proline contents of the yoghurt, with a concentration up to 300–500 mg/kg of the latter. Due to the activity of *Streptococcus salivarius subsp. thermophilus* carbamide contents of the yoghurt reduce to 10% of the original value. In the mixed cultures the phyla *Bifidobacterium lactis* and *Lactobacillus* show better growth and souring ratio than each separately what requires a symbiotic fermentation behaviour. Both phyla can be used alone, but they can be employed excellently together with other phyla, as well. In the experiments according to the individual species and mixtures that optimal temperature and duration were applied where the reproduction was the most intensive. For the mesophil species the ideal temperature is between 15–32 °C, for termophil bacteria 45–60 °C.

For the production of soured dairy products a milk supplied to a dairy company in Székelyland was used which was pasteurized at 78 °C for 50 sec. Temperature of the sample No. 1 was set to be 27 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis* and *Lactobacillus lactis subsp. cremoris* was added, subsequently the sample was incubated at 27 °C over 8 hours in a thermostat, then was refrigerated. After incubation the pH was 4.36. For the sample No. 2 the same cultures, temperature and duration were applied, therefore this sample could be regarded as repetition of the sample No 1. After incubation the pH was 4.43. Temperature of sample No. 3 was set to be 27 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus* was added. The sample was incubated at 27 °C over 7 hours, then refrigerated. After incubation the pH was 4.9. Temperature of sample No. 4 was set to be 28 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis subsp. lactis biovarn* was added. The sample was incubated at 28 °C for 7 hours, then refrigerated. After incubation the pH was 4.56. Temperature of sample No. 5 was set to be

28 °C and a pure culture mix of *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus diacetilactis* was added. The sample was incubated at 28 °C for 14 hours, then refrigerated. After incubation the pH was 4.56. Temperature of sample No. 6 was set to be 46 °C and a pure culture mix of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.21. Temperature of sample No. 7 was set to be 46 °C and a pure culture mix of *Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus* and *Streptococcus salivarius subsp. thermophilus* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.3. Temperature of sample No. 8 was set to be 46 °C and a pure culture mix of *Streptococcus thermophilus* and *Bifidobacterium lactis* was added. The sample was incubated at 46 °C for 6 hours, then refrigerated. After incubation the pH was 4.22. Sample No. 9 was the milk pasteurized at 78 °C for 50 sec, which was subsequently refrigerated. Sample No. 10 was the unpasteurized raw milk sample used as a control sample. In order to terminate bacterial activity, after the incubation the samples were immediately cooled down to –25 °C. Continued activity of lipase was not disturbing since during the analysis relative weight% of fatty acids was determined after transesterification, therefore free fatty acids formed due to lipase did not affect the result.

2.2 Determination of fatty acid composition

Sample preparation. A sample quantity containing approx. 0.5–1.0 g fat was destructed with 8–20 cm^3 of hydrochloric acid (37%) for 1 hour on hot water bath. After having cooled down, 7 cm^3 of ethanol was added. Lipids were extracted with 15 cm^3 diethylether and 15 cm^3 benzine (b.p.<60 °C), and the organic layers were combined. From a portion of this solution, containing approx. 150–200 mg fat, the solvents were removed at 80 °C under reduced pressure (a complete evaporation not necessary).

Transesterification. To the residue 4 cm^3 of 0.5 M sodium hydroxide methanol solution was added and boiled until all the fat drops disappeared (approx. 5 min), then 4 cm^3 of 14% boron trifluoride methanol solution was added, boiled for 3 min, finally 4 cm^3 of hexane, dried on water-free sodium sulphate, was added and boiled for 1 min, and the mixture was allowed to cool down. Saturated aqueous sodium chloride solution was added and after having separated the organic layer was collected into a 4 cm^3 vial containing water-free

sodium sulphate and was directly examined by gas chromatography.

Conditions of the gas chromatographic analysis. Instrument: Chrompack CP 9000 gas chromatograph. Column: 100 m×0.25 mm id, CP-Sil 88 (FAME) phase. Detector: FID 270 °C. Injector: splitter, 270 °C. Carrier gas: He, 235 kPa. Temperature program: 140 °C for 10 min; at 10 °C/min up to 235 °C; isotherm for 26 min. Injected volume: 0.5–2 µl.

2.3 Determination of conjugated linoleic acid contents

Lipid extraction. To a milk sample amount containing approx. 0.3 g fat 80 cm^3 of a 3:2 mixture of hexane and isopropanol (referred to as HIP) was added. The sample was dispergated in the liquid using a dispersion apparatus (Ultra-turrax T25 basic, manufactured by IKA) at 9.500 rpm for 2 min. The suspension was filtrated through a membrane filter (MN640W, 90 mm diameter). The filter was washed three times with 10 cm^3 of the HIP mixture and the organic layers were combined. 5 g of water-free sodium sulphate was added and the liquid was shaken up in order to eliminate water. The liquid was decanted and the solvents were removed at 80 °C under reduced pressure. The residue was washed into a 10 cm^3 volumetric flask with hexane.

Methylation. 0.5 cm^3 of the hexane solution obtained in the manner described above was taken into a 4 cm^3 capped vial and 0.5 cm^3 4 M sodium methylate methanol solution was added, it was shaken up and warmed to 50 °C and kept at this temperature for 30 min. Subsequently, 1 cm^3 of hexane and 1 cm^3 of water were added, it was shaken up, and after the layers have separated, 1 cm^3 of the organic layer was placed into a 5 cm^3 volumetric flask, to the aqueous layer 1.2 cm^3 of hexane was added, it was shaken up and 1 cm^3 of the hexanic layer was taken into the volumetric flask. This extraction with hexane was repeated twice more, the last time as far as it was possible the whole hexanic layer was collected, and the volumetric flask was filled up to 5 cm^3 with hexane, and the obtained solution was stored in a screw capped vial refrigerated until the analysis.

Conditions of the gas chromatographic analysis Instrument: Temperature program: column temperature 140 °C for 10 min; at 5 °C/min up to 235 °C; isotherm for 30 min. Injected volume: 2 µl. Other conditions are identical with those of described for determination of fatty acid composition.

3 Results

Short-chain fatty acids (C6–C12) of soured dairy product samples produced by adding various cultures are shown in *Table 1.*

Table 1: Short-chain fatty acid (C6–C12) contents of milk and dairy products

Various cultures	Fatty acid*			
	Caproic acid	Caprylic acid	Capric acid	Lauric acid
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	1.10	0.85	1.93	2.29
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	1.60	1.30	2.90	3.26
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	1.21	0.91	2.08	2.50
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	1.22	0.96	2.18	2.63
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	1.30	1.00	2.26	2.66
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	1.55	1.18	2.68	3.03
Lactobacillus delbrueckii subsp. bulgaricus,Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	1.27	0.96	2.14	2.50
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	1.11	0.90	2.05	2.43
Milk pateurized 78 °C, 50 min	1.40	1.10	2.46	2.90
Unpasteurized milk	1.14	0.92	2.13	2.56

*In relative weight% of fatty acid methyl esters.

Amount of miristic acid, palmitic acid, stearic acid and oleic acid which are the main fatty acid components is shown in *Table 2,* while amount of the essential and semi-essential fatty acids is shown in *Table 3.*

Having evaluated the results it was established that fatty acid composition of pasteurized milk and that of raw milk were practically identical within the limit of error of the measurement, and it can be established also for most of the cultures used, that the microorganisms did not produce any significant effect on the fatty acid composition. Apart from some minor discrepancies the data for each sample are practically identical, and although it is imaginable that carrying out the analyses with higher number of sample, significant differences could be obtained for some fatty acids, these differences would be, however, probably so slight that they would not affect nutritional value of soured dairy products.

Individually evaluating the fatty acids, it can be established that in the range of C6:0 and C15:0 the results practically coincide. For palmitic acid in case of the aroma and carbon dioxide producing cultures *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar* was found minimally deviating value whereas in each other cases the results were almost identical. For C16:1 and C17:0 this sample had the lowest value while in each other cases there was no difference between the samples. In case of stearic acid for the samples produced with the cultures *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus* (28 °C); *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus* (46 °C); *Streptococcus salivarius subsp. thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis* (46 °C) was found the lowest concentration whereas in the other cases the data practically coincided.

Evaluating all the samples, the biggest differences could be observed in case of elaidic acid where the lowest value was found to be 2.89% in case of the sample produced with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus* (46 °C), the highest value was found to be 7.58% for the sample produced with *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis subsp. biovar* (28 °C). In case of oleic acid the highest value was measured to be 28.60% for the sample produced with *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus,* while the oleic acid contents were of the lowest value with 23.58% for the sample produced with *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* and *Lactobacillus lactis*

subsp. lactis biovar (28 °C).

Table 2: Miristic acid, palmitic acid, stearic acid and oleic acid contents of milk and dairy products

Various cultures	Fatty acid*			
	Myristic acid	Palmitic acid	Stearic acid	Oleic acid
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	9.35	27.31	12.85	25.64
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	11.33	26.66	10.93	25.93
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	9.81	27.53	12.48	23.59
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	10.21	27.85	11.97	24.45
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	10.63	29.13	10.67	28.58
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	10.91	27.97	11.15	25.75
Lactobacillus delbrueckii subsp. bulgaricus,Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	9.60	28.00	12.32	24.50
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	9.53	27.11	13.17	24.13
Milk pateurized 78 °C, 50 min	10.88	27.73	11.50	24.30
Unpasteurized milk	10.07	27.70	12.50	25.02

*In relative weight% of fatty acid methyl esters.

Table 3: Linoleic acid, linolenic acid and conjugated linoleic acid contents of milk and dairy products

Various cultures	Fatty acid*		
	Linoleic acid	**Linolenic acid**	**Conjugated linoleic acid**
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 7 hours	2.13	1.67	0.49
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 27 °C, 8 hours	2.32	1.46	0.51
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 27 °C, 7 hours	2.10	1.62	0.49
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus lactis subsp. lactis biovar, 28 °C, 8 hours	2.10	1.61	0.50
Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, 28 °C, 14 hours	2.76	1.32	0.47
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus, 46 °C, 6 hours	2.20	1.45	0.46
Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus salivarius subsp. thermophilus Bifidobacterium lactis, 46 °C, 6 hours	2.01	1.61	0.48
Bifidobacterium lactis, Streptococcus salivarius subsp. thermophilus 46 °C, 6 hours	2.13	1.60	0.51
Milk pateurized 78 °C, 50 min	1.95	1.55	0.46
Unpasteurized milk	2.06	1.67	0.48

*In relative weight% of fatty acid methyl esters.

For all other cultures oleidic acid contents varied from 24.1 to 26.0%. For linoleic acid the sample obtained with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus* shows a somewhat deviating value, however, for all other fatty acids there is no substantial difference

in the concentration of the individual fatty acids. Proportion of elaidic acid in raw and pasteurized milk sample ranged between 5.31–5.51%, which barely changed due to the cultures. The highest value was reached with 7.58% for the sample *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus,* 27 °C, 7 h, whereas the lowest value with 2.89% for the sample *Lactobacillus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris,* 28 °C, 14 h.

Conjugated linoleic acid contents of raw milk was measured to be 0.48%, that did not change considerably either due to pasteurization or to the cultures used. From our examinations we could establish that CLA contents of raw milk were not lost after treatment with the cultures since soured products contained almost the same amount of CLA as the raw milk.

In summary, it can be said that due to the cultures we used and which are also commonly used in the dairy industry, original fatty acid composition of milk barely changed. Minimal discrepancies could be found for the individual fatty acids between the cultures, but these differences are so slight that it cannot be supposed that they could be supported also statistically by examinations carried out in higher numbers.

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